

Determination of Oxidative Stress, Vitamin A, E, C and β -Carotene Levels in the Tissues of *Luciobarbus esocinus* (Heckel, 1843)

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Abstract

In the study was investigated the levels of lipid peroxidation (malondialdehyde; MDA) as oxidative stress, vitamin E (VE), A (VA), C (VC) and β -carotene (β C) in liver, spleen, muscle and kidney tissues of *Luciobarbus esocinus*. The fish samples used in this study was caught from Keban Dam Lake (Elazığ-Turkey) at August. Analyzes were made in High Performance Liquid Chromatograph (HPLC). The data were analyzed with an Independent-Sample T Test in SPSS 21. It was determined that the levels of parameters analysed was generally higher in liver and spleen compared to kidney and muscle of female and male. The VE, VC and β C levels in liver and kidney were lower in female than male. The levels of VC in spleen were higher in male according to female. The VA level in muscle was lower in male than female, but the level of β C in this tissues was higher in male according to female. Moreover, the MDA level wasn't different according to sex.

Key words: *Luciobarbus esocinus*, Vitamins, Oxidative stress, Carotene

Luciobarbus esocinus (Heckel, 1843)'un Dokularındaki Oksidatif Stress, Vitamin A, E, C ve β -Karoten Seviyelerinin Tespiti

Öz

Bu çalışmada *Luciobarbus esocinus*'un karaciğer, dalak, kas ve böbrek dokularında oksidatif stres olarak lipid peroksidasyon (malondialdehit-MDA), vitamin E (VE), A (VA), C (VC) ve β -karoten (β C) seviyeleri araştırıldı. Çalışmada kullanılan balık örnekleri Ağustos ayında Keban Baraj Gölü'nden yakalandı. Analizler yüksek performanslı likit kromatografisinde yapıldı (HPLC). Veriler SPSS 21'de Independent-Sample T and Duncan Testi'nde analiz edildi. Genel olarak analiz yapılan parametrelerin düzeyleri erkek ve dişilerin böbrek ve kasma göre karaciğer ve dalağında daha yüksekti. Karaciğer ve böbrekteki VE, VC ve β C seviyeleri erkeklere göre dişilerde daha düşüktü. Dalaktaki VC seviyesi dişiye göre erkekte daha yüksekti. Kastaki VA seviyesi dişiye göre erkeklere daha düşüktü. Fakat bu dokudaki β C'nin seviyesi dişiye göre erkekte daha yüksekti. Bunlara ilave olarak bütün dokulardaki MDA seviyesi cinsiyete göre farklılık göstermedi.

Anahtar Kelimeler: *Luciobarbus esocinus*, Vitamin, Oksidatif Stres, Karoten

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1. Introduction

The reactive oxygen species (ROS) in biological system, natural by-products of aerobic metabolism, are essential in the physiological control of cell function. These compounds, highly deleterious, can rapidly react to form other molecules like peroxy and hydroperoxy radical. To cope with the continuous generation of ROS, there are adequate enzymatic and nonenzymatic antioxidant mechanisms. Oxidative stress (OS) occurs when the balance between the generation and the neutralization of ROS by antioxidant mechanisms within an organism is disrupted towards an overabundance of ROS [1-3].

Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and is associated with the presence of ROS. It is the biggest indicator of OS, and leads to the creation of lipid peroxides and other intermediates. The most common of these intermediates are 4-hydroxynonenal and malondialdehyde (MDA) [4,5]. The defence mechanisms against ROS consist of low-molecular-weight antioxidants like vitamin E, vitamin C and glutathione and antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and catalase [6,7]. Fish and crustacean that have rich source of polyunsaturated fatty acid lipids are more susceptible to the attack of ROS according to other aerobic organisms. For these reasons, excessive rise of ROS has also led to a decline in nutritional quality of these organisms [8,9]. Many studies have reported that ROS increase in the several situations such as exercise, pollution, infection, reproductive and starvation [8,10-12]. The body's complex antioxidant system is influenced by intake of non-enzymatic antioxidants such as VE, VC, VA and β C. These antioxidants is the major antioxidant in cell membranes and thus protects cell and organelle membranes from oxidation by reacting with organic free radicals[3,13,14].

Of the 2009 population of 6.7 billion people, 960 million were undernourished, and the human population is predicted to increase to 9.1 billion by 2050. It was reported that we have to turn to water and too much to land for additional food production. An important part of protecting the population and increasing the production of aquaculture species which are rich in micronutrients and omega-3 fatty acids is to know the features such as their biology, physiology and nutrition [15].

The demand of fish meat for the healthy life in the world is increasing day by day. Keban Dam Lake is a water resource located in the Eastern Anatolia Region and effective in meeting the fish needs. It was built around of Keban town in 45 km north of Elazığ province. It lies between 38 ° 37 'to 39 ° 20' northern latitudes and 38 ° 15 'to 39 ° 52' east longitudes in our region [16]. *L. esocinus* is the species of the cyprinidae family found in this dam lagoon. The meat is very tasty and the economic value is high. For this reason, *L. esocinus* is a major source of food for the people living here and income for the fishermen [17,18].

The present study was designed to compare the levels of Vitamins E, C, A, β -carotene and MDA in the tissues (liver, kidney, spleen, muscle) of *L. esocinus* according to sex. Thus, the obtained data will contribute to both that the food quality of *L. esocinus* and the basic level of MDA which is indicative of oxidative stress.

2. Materials and Methods

This study was carried out between 01-05 August at Firat University Aquaculture Faculty, Elazığ, Turkey. The *L. esocinus* used in this study was provided from Keban Dam Lake population.

In this study, the weight of fish were detected on a digital terrain with a sensitivity of ± 1 g. Weight of the fish was $1732,97 \pm 138,76$ g wet body for male, $1962,84 \pm 120,95$ g wet body for female. The among weight of *L. esocinus* not significantly different.

Dissolved oxygen (6.55 ± 0.98 mg L⁻¹), pH $8,09 \pm 0.19$ and water temperature $27,08 \pm 2,16$ °C were measured in the dam lake during the net fishing. The fish caught were brought to the laboratory in the ice. The liver, muscle, kidney and spleen were removed, wrapped in foil, kept at -20 °C until deep freezing analysis.

2.1. Determination of tissues vitamin C and MDA levels

An aliquot portion of (1,0 g) tissue samples were homogenized in a glass-glass homogenizer in mixture of 0.5 ml of HClO₄ (0.5 M), 4.5 ml distilled water. These samples were centrifuged at 4500 rpm for 5 min and supernatants were injected into HPLC system. Addition of acid was necessary to release the MDA and maintain the stability of vitamin C. The mobile phase was 30 mM KH₂PO₄ -Methanol (82.5+17.5, v/v %, pH 3.6). The flow rate of this phase was 1.2 mL min⁻¹. Chromatograms were monitored at 250 nm and injection volume was 20 µL. A Wakosil II 5C18 RS 5µm (150 x 4.6 mm SS, SGE, AUS) column was used at room temperature [19].

2.2. Determination of tissue vitamin A, E and β-carotene levels

The samples, ranging from 200 to 1000 mg weight, were homogenized in a glass-glass homogenizer in 1 ml of cold acetone. Homogenized tissue samples were transferred into polyethylene tubes and 2 ml ethanol was added to the tubes. After 0.3 ml n-hexane was filled into tubes for vitamins extractions, and they were centrifuged. N-hexane in tubes was evaporated with the nitrogen. Then the residues were solved in mobile phase (methanol: acetonitrile: chloroform; 47:42:11, v/v). Chromatograms were monitored at 326, 296 and 450 nm (vitamin A, E and β-carotene, respectively). Injection volume was set 50 µL. Techsphere ODS-2 packed column (5 mm particle) was used and flow rate was 1.0 mL min⁻¹[19].

The results in this study were expressed statistically by calculating the standard error of the mean (mean± SE). The data were performed with Duncan's test and independent-sample t-test, in SPSS 21 Brief Guide, Chicago, IL. The level of significance was set at $P < 0.05$.

3. Results

The data obtained from the study results was shown in Table1. Generally, the levels of MDA, VE, VC, VA and βC were higher significantly in liver and spleen in all tissues.

As a result of the analyzes made, VE level was found to be highest in liver ($76,78 \pm 5,16$ µg g⁻¹) of male fish. The levels of VC and βC was determined to be highest in spleen ($376,29 \pm 18,24$ µg g⁻¹, $250,78 \pm 14,28$ µg g⁻¹, respectively) of male fish. But, the level of VA was in close quantities ($22,18 \pm 2,16$ - $21,17 \pm 3,12$ µg g⁻¹) in the spleen of both male and female fish. The levels of MDA in liver

(1,25-1,08 $\mu\text{g g}^{-1}$) and kidney (0,96-1,03 $\mu\text{g g}^{-1}$) were higher than spleen (0,35-0,32 $\mu\text{g g}^{-1}$) and muscle (0,36-0,35 $\mu\text{g g}^{-1}$) both female and male.

Table 1. The levels of the MDA, vitamin E, vitamin C, vitamin A and β -carotene in liver, spleen, kidney and muscle tissue of *L.esocinus*.

Period	S	Parameters				
		VE ($\mu\text{g g}^{-1}$)	VC ($\mu\text{g g}^{-1}$)	VA ($\mu\text{g g}^{-1}$)	βC ($\mu\text{g g}^{-1}$)	MDA ($\mu\text{g g}^{-1}$)
Liver	♂	76,78±50,16x	102,16±8,16y	13,08±2,51y	123,19±4,16y	1,25±0,12x
	♀	59,19±4,08a	81,28±6,42b	10,39±1,98b	90,27±5,02b	1,08±0,24a
	P _L	***	**	*	***	-
Spleen	♂	47,18±3,16y	376,29±18,40x	22,18±2,16x	250,75±14,28x	0,35±0,05z
	♀	45,28±4,09b	300,16±20,15a	21,17±3,12a	243,18±16,28a	0,32±0,03b
	P _S	-	**	-	-	-
Kidney	♂	12,96±2,13t	59,25±5,28z	4,18±1,08z	53,28±4,28t	0,96±0,13xy
	♀	8,21±1,28d	32,18±4,16c	3,96±0,95cd	22,16±3,96d	1,03±0,20a
	P _K	**	**	-	***	-
Muscle	♂	16,29±1,18z	36,18±3,16t	3,29±1,09z	136,24±13,28z	0,36±0,03z
	♀	17,84±2,06c	34,21±2,95c	5,07±1,21c	76,16±6,95c	0,35±0,05b
	P _M	-	-	**	***	-
	P _♂	***	***	***	***	***
	P _♀	***	***	***	***	***

Note: the vitamin E (VE), vitamin C (VC), vitamin A (VA) and β -carotene (βC), sex (S). Istatistically comparison: P_L= for VE_♀-VE_♂, VC_♀-VC_♂, VA_♀-VA_♂, βC _♀- βC _♂, MDA_♀-MDA_♂, P_S= for VE_♀-VE_♂, VC_♀-VC_♂, VA_♀-VA_♂, βC _♀- βC _♂, MDA_♀-MDA_♂, P_K= for VE_♀-VE_♂, VC_♀-VC_♂, VA_♀-VA_♂, βC _♀- βC _♂, MDA_♀-MDA_♂, P_M= for VE_♀-VE_♂, VC_♀-VC_♂, VA_♀-VA_♂, βC _♀- βC _♂, MDA_♀-MDA_♂ (Independent-sample t-test), it wasn't used determiner in comparison of P_L, P_S, P_K, P_M. P_♀= a, b, c, d for VE_♀-VC_♀-VA_♀- βC _♀-MDA_♀ among the tissues P_♂= x, y, z, t for VE_♂-VC_♂-VA_♂- βC _♂-MDA_♂ among the tissues (Duncan's test).

The VE, VC and βC levels in liver and kidney were lower in female than male. The levels of VC in spleen were higher in male (376,29±18,24 $\mu\text{g g}^{-1}$) according to female (300,16±20,15 $\mu\text{g g}^{-1}$). The VA level in muscle was lower (3,29±1,09 $\mu\text{g g}^{-1}$) in male than female, but the level of βC in this tissues was higher (136,24±13,28 $\mu\text{g g}^{-1}$) in male according to female.

4. Discussion

Lipid peroxidation which is the result of interactions of lipid radicals and/or formation of nonradicals species by ROO^{*} is used to be a valuable indicator of the oxidative damage of cellular components [1,2,3]. Because of this reason, in the present study was investigated levels of MDA, as a secondary lipid peroxidation product. In our study was determined that the MDA level of *L. esocinus*'was generally higher in liver and kidney according to spleen and muscle of female and male. These findings are in agreement with a previous observation that was made by Paital and Chainy (2013), who found that the OS physiology markers were higher in liver in comparison to gills and abdominal muscle of *S. serrata* in year. Similarly, Verlecar *et al.* (2008) determined that digestive gland is specific tissues in variation of ROS level such as H₂O₂ and lipid proxidation of *P. viridis*. Moreover, in a lot of work on *A. leptodactylus* found that the hepatopancreas was more sensitive than the muscle and gills [22,23]. It is known that liver, the main digestive gland or hepatopancreas, contains fat-soluble vitamins, regulates the metabolism of the body and exhibits high oxygen consumption [24,25]. Thus, the generation of O₂^{-*} and H₂O₂ can be comparatively more in liver than other organs. Moreover, because the harmful

substances drained in blood accumulate in the kidney, chemical reactions can also increase in this organism.

It is very important in terms of controlling oxidative stress values as MDA to know the basic values of non-enzymatic antioxidants as VE, VC, VA and β C in tissues. For example, VE is a potent antioxidant with lipoperoxyl radical-scavenging activities. Vitamin C can protect biomembranes against peroxidative damage by trapping peroxy radicals in the aqueous phase before they can initiate LPO [23,26]. Vitamin A works as a good antioxidative agent as it neutralizes the free radicals [27]. The antioxidant activity of carotenoids when reacting with the $O_2^{\cdot-}$ anion through oxidation of this radical to oxygen arises in the capacity to prevent the formation of peroxides (H_2O_2/O_2^{2-}) and other reactive oxygen [28]. Bell *et al.* (2000) and Cavalli *et al.* (2003) also found that highly unsaturated fatty acids, that are vital components of cellular membranes, are particularly susceptible to attack by reactive oxygen radicals. For this reason, as a result of the investigations, it has been determined that metabolic activity and oxidative stress increase in direct proportion. Our study was found that the levels of both analyzed antioxidants and MDA were higher in tissues with high metabolic activity as liver and kidney.

In terms of healthy nutrition, the aliment value of the foods is very important. For this reason, people especially prefer fish that live under natural conditions. Many studies have identified nutritional values of aquatic organisms. It was determined by Arslan *et al.* (1997) the level of VE in muscle tissues of *O. mykiss* ($711.3 \mu\text{g } 100\text{g}^{-1}$), *C. carpio* ($787.4 \mu\text{g } 100 \text{g}^{-1}$) and *C. trutta* ($656.4 \mu\text{g } 100 \text{g}^{-1}$) in the study conducted in the same region. Moreover, Köprücü and Özdemir (2002) identified that A₂, VC and VE level in muscle of cultured rainbow trout (*O. mykiss*) was $12.26 \pm 0.02 \mu\text{g g}^{-1}$, $17.54 \pm 0.56 \mu\text{g g}^{-1}$, $8.73 \pm 0.03 \mu\text{g g}^{-1}$, respectively. Vitamin E and VC values were also determined in studies performed on different species in different regions. For example, some species with VE value have been identified as *O. mykiss* ($5 \mu\text{g g}^{-1}$), *T. thynnus* ($6 \mu\text{g g}^{-1}$) (21), *I. punctatus* ($24.2 \mu\text{g g}^{-1}$), *C. harengus* ($11 \mu\text{g g}^{-1}$), *O. mykiss* ($18.5 \mu\text{g g}^{-1}$), *P. clarkii* ($10 \mu\text{g g}^{-1}$) and *C. virginica* ($31 \mu\text{g g}^{-1}$) [33]. It was determined that VE level in muscle of cyprinidae spp. was $630 \text{ mg } 100 \text{g}^{-1}$. Additionally, this level was found as $9.95 \mu\text{g g}^{-1}$ in *A. grypus* [34]. The differences between the data may be due to differences in species, seasonality and nutrient quality of the living environment, as well as differences in the extraction method and test conditions used.

This study represents the first report of oxidative stress and some nonenzymatic antioxidant defence system (as VE, VC, VA, β C) of *L. esocinus* in Keban Dam Lake (Turkey). In addition, this study revealed the nutritional value of *L. esocinus*. Thus, people living in this region were encouraged to go for better quality protein with less money.

5. References

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